

replaced by
Article 31

Claims

1. Protein L having the ability to bind to the light chains of immunoglobulins, characterized in that the protein L has the following amino acid sequence:

B1

Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser
1 5 10 15

Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser
20 25 30

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu
35 40 45

Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr
50 55 60

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
65 70 75 80

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
85 90 95

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
100 105 110

Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu
115 120 125

Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr
130 135 140

B3

Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro
145 150 155 160

Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys
165 170 175

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu
180 185 190

Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr
195 200 205

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
210 215 220

B4

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
 225 230 235 240

5 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
 245 250 255

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
 260 265 270

10 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
 275 280 — B5 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
 290 295 300

Glu

15

and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties.

20 2. DNA-sequence, characterized in that it codes for the protein according to Claim 1 and has the following nucleotide sequence:

GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA	48
25 GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA ACC	96
ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA	144
GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT	192
30 GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA	240
AAA GAA AAA ACA CCA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA	288
AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA	336
ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA	384
35 AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT	432
ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA	480
AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA	528

ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576
GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624
5 GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 672
AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720
AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768
10 ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA 816
GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864
ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912
15 GAA TAATAA 921

3. A hybrid protein, characterized in
that it includes one or more of the B1-B5-domains ac-
20 cording to Claim 1 which bind to the light chains in
immunoglobulins of all classes, and domains which bind
to heavy chains in immunoglobulin G.

4. A hybrid protein according to Claim 3, char-
acterized in that the domains which bind to
heavy chains in immunoglobulin G are chosen from among
the C1- and C2-domains in protein G or from among any
other functionally similar proteins which bind to heavy
chains in immunoglobulin G, and variants, subfragments,
30 multiples or mixtures thereof having the same binding
properties.

5. A hybrid protein according to Claim 4, char-
acterized in that the hybrid protein has the
35 following amino acid sequence:

Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser
 1 5 10 15

5 Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser
 20 25 30

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu
 35 40 45

10 Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr
 50 55 60

Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
 65 70 75 80

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
 15 85 90 95

Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
 100 105 110

Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu
 115 120 125

20 Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr
 130 135 140

Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro
 145 150 155 160

25 Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys
 165 170 175

Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu
 180 185 190

Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr
 195 200 205

30 Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly
 210 215 220

Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
 225 230 235 240

35 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
 245 250 255

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
260 265 270

5 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
275 280 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
290 295 300

10 Glu Pro Met Asp Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys
305 310 315 320

Gly Glu Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val
325 330 335

Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr
15 340 345 350

Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Glu Val Ile
355 360 365

Asp Ala Ser Glu Leu Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile
370 375 380

20 Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr Lys Ala Val Asp Ala
385 390 395 400

Glu Thr Ala Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val
405 410 415

25 Asp Gly Val Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr
420 425 430

Glu Met

30 and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties.

6. DNA-sequence, characterized in that it codes for a protein according to Claim 5 and has the 35 following nucleotide sequence:

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GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA 48
 GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA AGC 96
 ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA 134
 GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT 192
 5 GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 240
 AAA GAA AAA ACA CCA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 288
 AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA 336
 10 ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA 384
 AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT 432
 ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA 480
 AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA 528
 15 ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576
 GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624
 GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 672
 20 AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720
 AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768
 ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA 816
 GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864
 25 ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912
 GAA CCC ATG GAC ACT TAC AAA TTA ATC CTT AAT GGT AAA ACA TTG AAA 960
 GGC GAA ACA ACT ACT GAA GCT GTT GAT GCT GCT ACT GCA GAA AAA GTC 1008
 30 TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT GAC GGT GAA TGG ACT TAC 1056
 GAC GAT GCG ACT AAG ACC TTT ACA GTT ACT GAA AAA CCA GAA GTG ATC 1104
 GAT GCG TCT GAA TTA ACA CCA GCC GTG ACA ACT TAC AAA CTT GTT ATT 1152
 AAT GGT AAA ACA TTG AAA GGC GAA ACA ACT ACT AAA GCA GTA GAC GCA 1200
 35 GAA ACT GCA GAA AAA GCC TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT 1248
 GAT GGT GTT TGG ACT TAT GAT GAT GCG ACT AAG ACC TTT ACG GTA ACT 1296
 GAA ATG TAATAA 1308

7. DNA-sequence, characterized in that it codes for a protein according to Claim 3 and 4.

8. DNA-sequence, characterized in that 5 it hybridizes to the DNA-sequence of Claim 2, 6 or 7 under conventional conditions and codes for a protein which has the same binding properties as the protein according to any one of Claims 1 and 3-5.

10 9. A plasmid vector, characterized in that it includes a DNA-sequence according to any one of Claims 2 and 6-8, preferably the vector pHDLG or pHDL.

15 10. A host cell, characterized in that it is transformed with the hybrid plasmid according to Claim 9, in particular a host which belongs to the species E. coli, particularly E. coli LE392, or Bacillus subtilis, Saccharomyces cerevisiae, preferably Id. Ref. DSSM E. coli LE392 pHDL and E. coli LE392/pHDLG respectively.

25 11. A method for producing a protein according to Claims 1 and 3-5, characterized by cultivating a host cell according to Claim 10 under suitable conditions; accumulating the protein in the culture or lysing the cells and extracting the protein therefrom.

30 12. A reagent apparatus for binding, separating and identifying immunoglobulins, characterized in that it includes a protein according to any one of Claims 1 and 3-5.

35 13. A composition, characterized in that it includes a protein according to any one of Claims 1 and 3-5, and optionally additives or carriers.

14. A pharmaceutical composition, characterized in that it includes a protein according to any one of Claims 1 and 3-5, and optionally a pharmaceutically acceptable carrier or extender.